

Thlaspi caerulescens on nonmetalliferous soil in Luxembourg: ecological niche and genetic variation in mineral element composition

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Summary

- Forty-seven populations of *Thlaspi caerulescens* in Luxembourg were characterised for population size, soil mineral element composition and other habitat characteristics. Foliar concentrations of eight elements were assessed in 15 populations in the field and in eight populations cultivated in zinc (Zn)-cadmium (Cd)-nickel (Ni)-enriched soil.
- *T. caerulescens* favoured stony soil developed on steep, south-facing Emsian shale outcrops. All soil samples were nonmetalliferous. Soil pH ranged from 4.2 to 6.9. Field-growing plants had very high concentrations of heavy metals in the leaves (Zn, 3000–13 000 mg kg⁻¹; Cd, 11–44 mg kg⁻¹; Ni, 38–473 mg kg⁻¹). Positive soil-plant correlations existed for Zn and Mn.
- In cultivation, significant genetic variation was found for biomass and six of eight mineral elements. For Cd and Zn, variation range among 48 half-sib families was two-fold (Cd, 183–334 mg kg⁻¹; Zn, 8030–16 295 mg kg⁻¹). Most of the variation occurred among populations, consistent with the selfing mating system of those populations. There was a tight Zn–Cd genetic correlation ($r = +0.83$, $P < 0.0001$).
- The significance of the results to the conservation of *T. caerulescens* in Luxembourg is briefly discussed.

Key words: cadmium (Cd), ecological niche, genetic variation, hyperaccumulation, nickel (Ni), phytoremediation, *Thlaspi caerulescens*, zinc (Zn).

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Introduction

Thlaspi caerulescens has become one of the best-studied heavy metal hyperaccumulators (Pollard *et al.*, 2002; Assunção *et al.*, 2003b; Cobbett, 2003; Macnair, 2003). The species was once considered an absolute metallophyte, restricted to calamine (i.e. Zn–Cd enriched) and serpentine soil (Reeves & Brooks, 1983; Lloyd-Thomas, 1995). Its occurrence on nonmetalliferous substrates has been more recently acknowledged, possibly because such populations are extremely rare in Great Britain, where the species ecology has been most thoroughly studied (Ingrouille & Smirnoff, 1986; García-Gonzalez & Clark, 1989; Baker *et al.*, 1994). Nonmetallicolous populations are apparently not rare in continental Europe (Koch *et al.*,

1998), where they often occur in mountain grassland (Ellenberg, 1988). Reeves *et al.* (2001) were able to locate a relatively large number of populations on nonmetalliferous substrate in France. They also showed that these accumulated Zn, Cd and Ni to high concentrations on their native, metal-poor soil.

It is now clear that *T. caerulescens* is not uniform for metal accumulation capability (Pollard *et al.*, 2002). Variation in Zn accumulation was documented between 'ecotypes' (Meerts & Van Isacker, 1997; Assunção *et al.*, 2003c; Frérot *et al.*, 2003; Roosens *et al.*, 2003) and between populations within ecotypes (Pollard & Baker, 1996; Meerts & Van Isacker, 1997; Escarré *et al.*, 2000; Lombi *et al.*, 2000). Significant variation within populations was also occasionally reported (Pollard &

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4 Baker, 1996; Meerts & Van Isacker, 1997; Escarré *et al.*, 2000). The first published characterisation of metal accumulation by nonmetallicolous populations in controlled conditions was apparently performed by Meerts & Van Isacker (1997). They showed that nonmetallicolous populations from Luxembourg had much higher Zn accumulation capacity compared with metallicolous populations from Belgium, a result later confirmed by Assunção *et al.* (2003c) and Meerts *et al.* (2003). One nonmetallicolous population from Luxembourg has recently been characterised from a physiological point of view (Assunção *et al.*, 2001; Assunção, 2003a).

5 6 Compared with metallicolous populations, the ecological conditions in which *T. caerulescens* occurs outside metalliferous sites are poorly known. The extent and structure of variation in metal hyperaccumulation existing in noncontaminated sites is also poorly known. Clearly, a better knowledge of population ecology of *T. caerulescens* on normal soil might provide insight into the ecological and evolutionary significance of metal hyperaccumulation in circumstances where it is apparently not related to metal tolerance.

In this paper, we investigate population ecology of *Thlaspi caerulescens* in the Grand-Duchy of Luxembourg. Our objectives are first to characterise the distribution of the species in relation to a large number of habitat parameters, and second to assess the extent and partitioning of genetic variation in mineral element composition. Based on these data, we examine the factors explaining variation in mineral element composition of field growing plants. The significance of the results to the conservation of *T. caerulescens* in Luxembourg is briefly discussed.

Materials and Methods

Field work

7 In spring 2002, at the time of flowering of *T. caerulescens*, the distribution area of *T. caerulescens* in Luxembourg, as mapped in the 'Atlas de la flore belge et luxembourgeoise' (Assunção, 1979), was extensively explored. All populations are located in the Ardennes phytogeographical district as defined by Lambinon *et al.* (1992), in a region locally known as 'Oesling'. A total of 47 populations were thus located. For each population, the following data were obtained: population size (6 classes based on the number of flowering individuals: 1–10; 11–50; 51–100; 101–499; 500–999; ≥ 1000), altitude, slope and aspect. The most abundant accompanying species were recorded and a soil sample was collected (bulk sample based on five independent cores; depth: 0–5 cm). Geological bedrock was determined based on the 'Carte géologique du Grand-Duché du Luxembourg' (Lucius, 1949). In 15 populations, five rosettes were collected for mineral element analyses (each rosette was taken close to a soil core), and seeds were collected in May 2002 on 10 maternal plants.

Cultivation in controlled conditions

The seeds were sown in September 2002 in Petri dishes placed in a growth chamber. Two weeks after emergence, the seedlings were transferred for a further 2 wk in a glasshouse in pots filled with garden compost. Then, eight populations with a sufficiently high number of even-sized seedlings in at least six maternal families were randomly selected for cultivation in metal-enriched soil. The experimental substrate consisted of arable soil supplemented with Zn (1000 mg kg⁻¹), Cd (30 mg kg⁻¹), and Ni (30 mg kg⁻¹) as monometallic salts (ZnO; 3(CdSO₄)·8H₂O; NiSO₄·6H₂O, respectively). Zinc oxide was used in lieu of zinc sulphate because the latter results in substantial soil acidification that is not well tolerated by Luxembourg populations (Meerts *et al.*, 2003); zinc oxide is highly available to *T. caerulescens* (Whiting *et al.*, 2001; Meerts *et al.*, 2003). The mineral element composition of the substrate prior to contamination was as follows (in mg kg⁻¹, extraction with ammonium acetate-EDTA 1 N pH 4.65): Ca: 2147; Mg: 13; K: 170; Fe: 302; Mn: 27; Zn: 9; Cd: < 1; Ni: 1. After metal salt addition, 80% of Zn and 100% of Cd and Ni were recovered by the same extraction method. The metal salts were thoroughly mixed up with soil for each pot separately (the pots were 11 cm in diameter and contained 225 g dry soil). One seedling was transplanted into each pot. The 240 pots (eight populations × six families × five replicates) were arranged in five blocks. Within each block, pots were randomised every 2 months. The pots were placed in an unlit glasshouse. Temperature was not allowed to drop below 5°C but was not otherwise controlled. The pots were watered with deionised water as necessary. In May 2003, four blocks were harvested (above-ground parts only; the fifth block was not harvested in order to obtain seed). No plant showed toxicity symptoms (chlorosis, ...). The material was rinsed in deionised water, dried at 60°C for 48 h, and weighed to the nearest 0.001 g.

Plant and soil analyses

Dried soil samples were sieved with a 2-mm mesh. The proportion of stones (i.e. particles > 2 mm) was determined by weighing. pH was measured with a glass electrode on a water-saturated soil sample. Mineral elements were extracted with ammonium acetate-EDTA 1 N (pH 4.65) for 30 min (10 g dry soil in 50 ml) (Cottenie *et al.*, 1982). Supernatant was filtered and analysed by ICP-OES (Varian Vista MPX) for the following nine elements: Ca, Mg, P, K, Fe, Mn, Zn, Cd, Ni. In 15 samples, carbon content was determined with a Strohlein carbon analyser, and a granulometric analysis was performed after Delecour & Kindermans (1980).

Plant samples (rosettes) collected in the field were carefully cleaned to remove any adhering soil particles. Old (i.e. withered or chlorotic) and otherwise damaged leaves were discarded. For both field and cultivated plants, dried plant

samples (c. 0.5 g) were mineralised with a Tecator digester in a mixture of nitric and perchloric acid (2 : 1). The samples were then analysed by ICP-OES for the same elements as in soil, except for P.

Data analysis

Ecological amplitude of *T. caerulea* populations was expressed graphically by means of box-and-whiskers plots and distribution frequency histograms.

Variation of mineral element composition of cultivated plants was analysed by partially nested ANOVA, with families nested within populations. The block*family within population interaction was used as the error term in the analysis since each maternal family was represented by a single replicate in each block. Block, population and family were treated as random factors. Correlations between mineral composition of plants and their native soil were examined for both field-collected and cultivated plants. All analyses were performed with Statistica 6 (StatSoft, 2003).

Results

Fig. 1 shows the location of all populations investigated. Most populations are restricted to the river valleys of the Ardennes massif (River Sûre and tributaries), at altitudes ranging from 235 to 495 m asl (mean \pm SD: 350 \pm 63 m asl).

Population size

Population size (Fig. 2a) had an asymmetrical distribution, with a majority of populations counting fewer than 100 individuals. A few populations, however, had more than 1000 flowering plants.

Slope, aspect and bedrock

T. caerulea was most often found on steep (mostly > 30°), SE- to SW-facing slopes (Fig. 2b,c). From a geological viewpoint, all populations occurred on Lower Devonian shale (from Upper Siegenian to Middle Emsian). By far the most

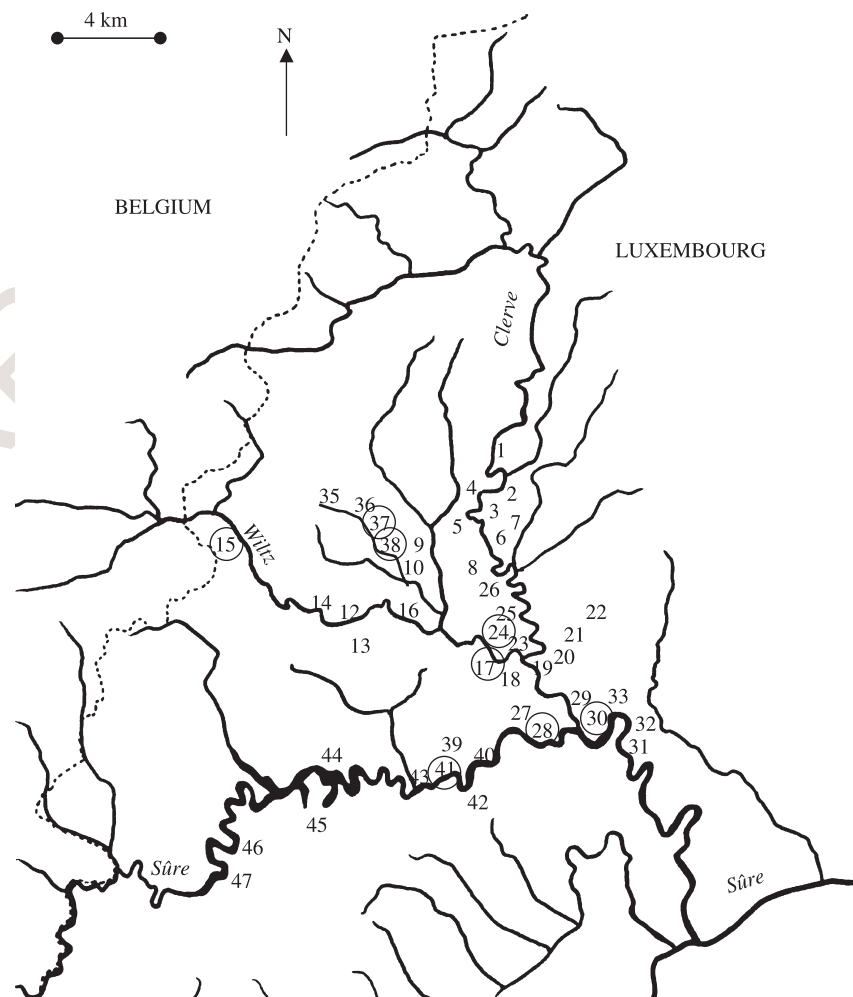


Fig. 1 Location of the 47 populations of *Thlaspi caerulea* studied. River valleys are indicated.

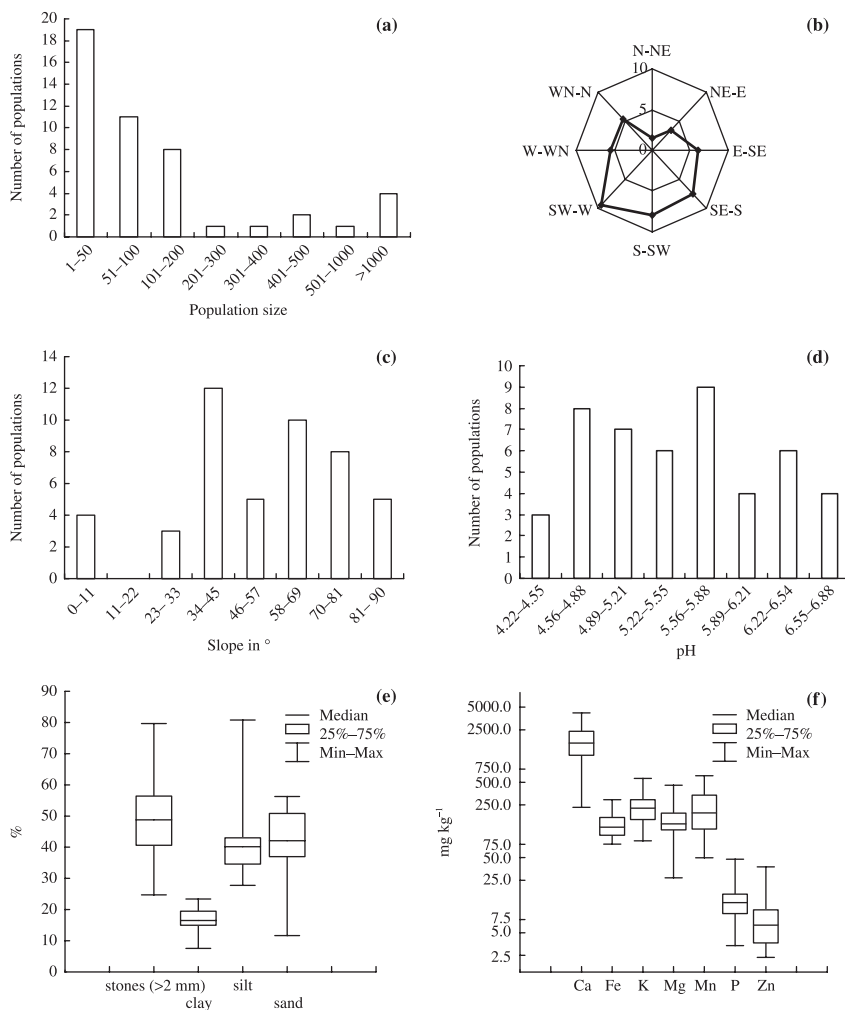


Fig. 2 Ecology of 47 populations of *Thlaspi caerulescens* in Luxembourg. (a) population size (number of flowering individuals) (b) aspect (c) slope (d) soil pH (e) soil granulometry and stone percentage (f) concentration of seven elements in the soil (extraction with ammonium acetate-EDTA 1 M pH 4.65)

frequent substrates were the 'schistes de Stolzembourg' (19 populations) and the 'schistes de Wiltz' (17 populations) (E1a and E3, respectively, on the 'Carte géologique du Grand-Duché du Luxembourg' (Lucius, 1949)). There, *T. caerulescens* occurred on shallow, often skeletal, soils containing 25%–80% stones (Fig. 2e). The soils developed on those bedrocks were relatively light, often with more than 38% sand and less than 20% clay (Fig. 2e). They had moderate carbon content (2.7–7.5%).

Soil pH showed extensive variation range, from 4.2 to 6.9, with a bimodal distribution (peaks at 4.8–5.2 and at 5.6–6.0) (Fig. 2d). Fig. 2(f) shows the variation in soil mineral element concentrations (seven elements) across the 47 sites. The wide variation range in pH is in line with a similarly wide variation in the concentrations of Ca (*c.* 230 mg kg⁻¹–4200 mg kg⁻¹) and Mg (25–460 mg kg⁻¹). All soils had low concentrations of heavy metals, which are within the range of nonmetalliferous soils. Cd and Ni were close to detection limits (< 1 mg kg⁻¹). For Zn, however, a few samples exceeded the upper value of the normal range (18 mg kg⁻¹ extractable Zn (Cottenie *et al.*,

1982)). Extractable P was consistently very low in these soils (Fig. 2f) as compared to reference values for normal soils (Cottenie *et al.*, 1982).

Accompanying flora

Most populations occurred in open herbaceous vegetation, often near the fringe of Oak-hornbeam woodland and coppice. A total of 186 species of flowering plants were recorded with an average of 24 species per site. Those 16 species most frequently found with *T. caerulescens* (i.e. more than 40% of the relevés) are: *Galium mollugo* (79%)^(B), *Teucrium scorodonia* (67%)^(A), *Fragaria vesca* (65%), *Lapsana communis* (58%), *Hypericum perforatum* (58%), *Euphorbia cyparissias* (55%)^(B), *Cytisus scoparius* (55%)^(A), *Vicia sepium* (51%), *Senecio jacobaea* (48%)^(B), *Prunus spinosa* (48%)^(B), *Arrhenatherum elatius* (48%)^(B), *Quercus robur* (46%), *Epilobium collinum* (46%)^(A), *Sedum reflexum* (44%), *Digitalis purpurea* (44%)^(A), *Rumex acetosa* (41%). A number of these species (marked^(A)) are acidity indicators, and others are indicators of neutral

Table 1 Mineral element concentrations in field growing *Thlaspi caerulescens* plants and in corresponding soil and plant–soil concentration factors

		Ca	Mg	K	Fe	Mn	Zn	Cd	Ni
Plant in situ	Mean	18041	3268	16831	674	178	7257	31	178
	SD	2672	820	5756	364	92	3748	9	119
Soil in situ	Mean	1788	159	235	143	238	8.6	< 1	< 1
	SD	839	73	102	57.6	149.4	7.4	–	–
P/S in situ	Mean	12.7	25.3	74	4.6	0.8	1029	> 31	> 178
	SD	6.2	13	31.5	2.9	0.4	525	–	–

Mineral element concentrations (mg kg⁻¹)

Soil (extraction with ammonium acetate-EDTA 1 M pH = 4.65)

(n = 15).

Table 2 Correlation between mineral element composition of *Thlaspi caerulescens* in the field and mineral element composition of local soil

	Ca	Mg	K	Fe	Mn	Zn
Soil–plant correlation	+0.33	–0.16	+0.41	+0.16	+0.73**	+0.50*

(n = 15).

Correlations for Cd and Ni are not calculated because soil concentrations of these metals are close to the detection limit.

***P* < 0.01; **P* < 0.05 (one-tailed tests).

or basic soil (marked^(B)). The high frequency of *Sedum reflexum*, a xerophilous, chasmophytic species is in line with the occurrence of shale outcrops.

Mineral composition of field-growing plants

Table 1 shows foliar concentrations of eight elements measured in field-growing plants. For Zn, Cd and Ni, observed values were well above the normal range for nonaccumulating plants (Zn: 3000–13000 mg kg⁻¹, Cd: 11–44 mg kg⁻¹, Ni: 38–473 mg kg⁻¹). Zn concentrations exceeded the conventional hyperaccumulation threshold (10 000 mg kg⁻¹) in four of 13

populations. Plant/soil concentration ratio was much higher for Zn (1030) compared with all other elements.

There were positive correlations between the element concentrations in plants and in soil in the case of Mn ($r = +0.73$, $n = 15$, $P < 0.01$, one-tailed) and Zn ($r = +0.50$, $n = 15$, $P < 0.05$, one-tailed) (Table 2). There was a significant, negative correlation between soil pH and Mg in plants ($r = -0.66$, $n = 15$, $P < 0.05$).

Variation of mineral element composition in controlled conditions

A significant added variance component at the population level was found for biomass and for all mineral elements except Fe, Mn and Ni (Table 3). Variance percentage accounted for by the population effect ranged from 10.4% (Ca) to 27.2% (biomass). Ratio of the highest to the lowest population mean value ranged from 1.2 (biomass) to 1.43 (Zn). Populations 1, 2 and 8 had the highest concentrations of Zn and Cd and population 5 had the lowest concentrations of these elements (Fig. 3).

Variance among family within population was significant for Cd and Zn only (Table 3). This could be accounted for by significant variation among family within only two populations. Population 1 had significant family variance for both Zn and Cd and population 6 for Zn only (data not shown). Variance percentage accounted for by the family effect was

Table 3 Analysis of variance of biomass and mineral element composition in *Thlaspi caerulescens* from Luxembourg cultivated in soil enriched in zinc (Zn)–cadmium (Cd)–nickel (Ni): percentage of total variance accounted for by block, population and family within population

	Shoot mass	Ca	Cd	Fe	K	Mg	Mn	Ni	Zn
Block	0	28.6***	9.7***	1.9	3.3*	2.8*	0	0	11.2***
Population	27.2***	10.4**	15.9**	4.6	11.8*	13.3**	0	5.9	19.3***
Family (population)	4.9	0.3	15**	7.6	0	1.7	0	0.8	10.2*
Block*population	0	0	0	0	0	0	2.9	1.2	0
Block*family (population) = Error	67.9	60.7	59.4	85.8	84.9	82.2	97.1	92.1	59.3

Six maternal families in each of eight populations. Block, population and family were treated as random factors. ****P* < 0.001; ***P* < 0.01; **P* < 0.05.

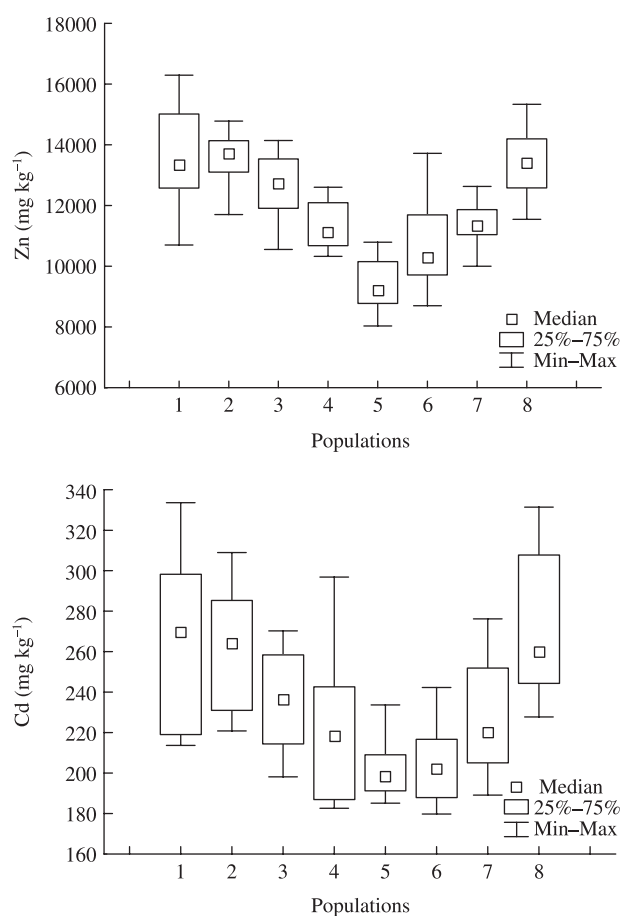


Fig. 3 Foliar concentrations of zinc (Zn), cadmium (Cd) in eight populations of *Thlaspi caerulescens* (six half-sib families per population) cultivated on soil spiked with 1000 mg kg⁻¹ Zn, 30 mg kg⁻¹ Cd and 30 mg kg⁻¹ Ni: range of family mean values. Populations numbered 1–8 in this figure correspond to the following numbers on the map (Fig. 1): 1, 15; 2, 17; 3, 24; 4, 28; 5, 30; 6, 37; 7, 38; 8, 41.

always smaller compared with variance among populations. For Cd and Zn, variation range among the 48 maternal family mean values was more than two-fold (Cd, 183–334 mg kg⁻¹; Zn, 8030–16295 mg kg⁻¹) (Fig. 3).

Table 4 Correlation (Pearson's *r*) between mineral element concentrations and shoot mass in *Thlaspi caerulescens* plants grown in controlled conditions

Shoot mass	Ca	Mg	K	Fe	Mn	Zn	Cd	
Ca	-0.32***							
Mg	-0.24***	+0.52***						
K	-0.36***	+0.48***	+0.59***					
Fe	-0.29***	+0.28***	+0.36***	+0.34***				
Mn	-0.08	+0.11	+0.11	+0.05	+0.26***			
Zn	-0.07	+0.45***	+0.58***	+0.32***	+0.07	-0.01		
Cd	-0.09	+0.39***	+0.52***	+0.32***	+0.08	-0.04	+0.83***	
Ni	+0.05	+0.18*	+0.32***	+0.18*	-0.12	-0.11	+0.40***	+0.33***

(*n* = 192). ****P* < 0.001; ***P* < 0.01; **P* < 0.05.

There were a number of significant correlations between different elements in the plants (Table 4). By far the most significant one was the Zn–Cd correlation ($r = +0.83$, $P < 0.001$, Fig. 4). The Zn–Ni correlation was also significant ($r = +0.41$, $P < 0.001$).

Discussion

Ecological profile: climate and soil physical properties

All populations of *T. caerulescens* in Luxembourg are restricted to river valleys of the Ardennes massif in a region locally known as 'Oesling'. Compared with the cold, rainy climate of the Ardennes plateaux, river valleys of Oesling have milder (8.5–9°C), less rainy (750–800 mm yr⁻¹) climate (Lahr, 1950). A striking result is the marked preference of the species for steep (mostly > 30°), mostly south-facing slopes. These conditions, in addition to shallow soil rich in dark-coloured shale fragments, certainly result in a warm microclimate. The species thus appears to be heliophilous and thermophilous in the studied region. The shallow soils where it occurs, overlying shale outcrops on steep slopes and with a high percentage of stones are certainly well drained. Actually, these soils often dry out in summer (pers. obs.). It is striking that the physical soil conditions and microclimate of the nonmetallicolous ecotype of *T. caerulescens* in Luxembourg are reminiscent of the conditions that prevail on mine deposits in E Belgium, where the species is represented by the metallicolous ecotype. Natural zinc outcrops may also have had similar conditions, which may have facilitated the evolution of metallicolous populations from neighbouring populations on normal soil.

Ecological profile: soil chemistry

Soil pH spans a wide range of values (4.2–6.9), corresponding to a similarly wide range in extractable calcium (200–4200 mg kg⁻¹). This is probably due to the sporadic occurrence of clay or calcium carbonate nodules in the Emsian shale (Reichling, 1968). Lambinon *et al.* (1992) consider *T. caerulescens* ssp. *caerulescens* as acidophilous. Even though most populations

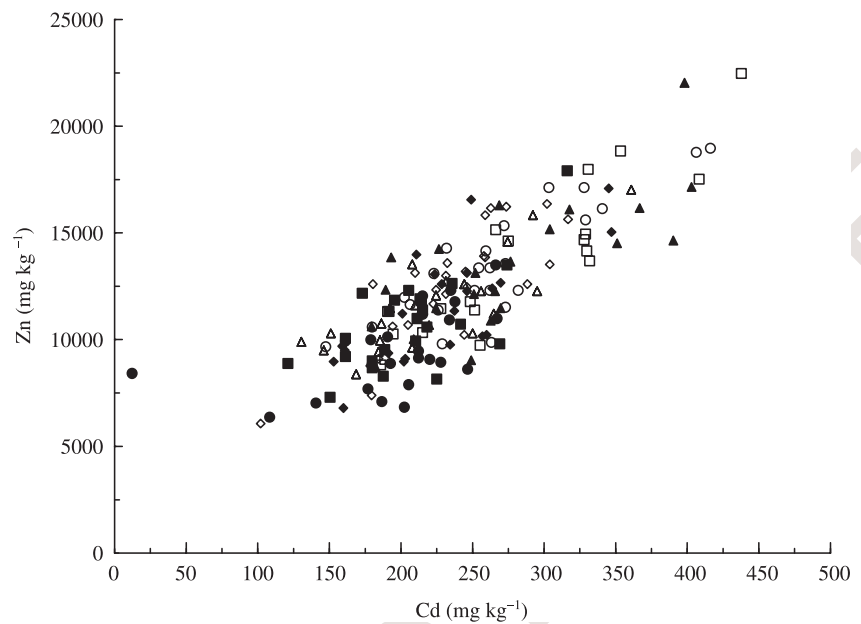


Fig. 4 Correlation between foliar concentrations of zinc (Zn) and cadmium (Cd) in eight populations of *Thlaspi caerulescens* from Luxembourg cultivated on soil spiked with 1000 mg/kg Zn, 30 mg kg⁻¹ Cd and 30 mg kg⁻¹ Ni. Population symbols: 1, open circle; 2, open square; 3, open diamond; 4, open triangle; 5, solid circle; 6, solid square; 7, solid diamond; 8, solid triangle.

do occur on markedly acidic soil, the species is by no means restricted to them, also occurring in neutral soil. Compared with reference values for the extraction method (Cottenie *et al.*, 1982), the concentrations of most nutrients lie within the normal range, except for P that is consistently in the lowest range of reference values. As a short-lived species adapted to unproductive soil, *T. caerulescens* can be ascribed to the ruderal stress-tolerant strategy of (Grime *et al.*, 1988). Most, if not all populations of *T. caerulescens* in Luxembourg grow on soil with background values of heavy metals. However, Zn concentrations of a few samples exceeded the upper limit of the normal range (> 18 mg kg⁻¹), although they were still one order of magnitude lower than values expected in calamine soil.

Flora and vegetation

T. caerulescens occurs in open plant communities with relatively low productivity due to a combination of thin soil, excessive drainage and limiting phosphorus. A large number of populations occur on road banks, where yearly mowing precludes invasion by woody species. Our observations suggest that mowing must be regarded as a suitable management for *T. caerulescens* in Luxembourg. However, too early mowing (i.e. before the end of May) may occasionally preclude the species from completing its life cycle (pers. obs.).

In Central Europe, *T. caerulescens* is regarded as a member of mountain, mesic grassland (phytosociological alliances of the Trisetio-Polygonion, Arrhenatherion, Geranio-Trisetion) (Oberdorfer & Müller, 1983; Ellenberg, 1988; Rothmaler *et al.*, 1990). Although we did not perform a detailed phytosociological analysis, it is clear that *T. caerulescens* vegetations in Luxembourg are quite different. Our results are reminiscent of the vegetation in which *T. caerulescens* occurs

in the Morvan mountains (E France), c. 400 km south of Luxembourg. In that region *T. caerulescens* is a characteristic species of the Trifolio-Teucrion scorodoniae comprising thermophilous forest fringes on thin, base-poor soil (Robbe, 1993). The flora accompanying *T. caerulescens* in Luxembourg is clearly heterogeneous with respect to pH indicator values. This is certainly related to the heterogeneity of Emsian shale with respect to calcium carbonate content and fits in well with the widely varying pH of these soils.

Mineral element composition in the field

Zn, Cd and Ni concentrations in field growing plants were very high, considering the low concentrations of those elements in soil. Reeves *et al.* (2001) already reported similarly high values for populations of *T. caerulescens* on normal soil in France. Our results once again confirm that *T. caerulescens* is constitutively hyperaccumulator of Zn (Assunção, 2003a). The concentrations of Cd, although not exceeding the conventional hyperaccumulation threshold (100 mg kg⁻¹), are high (11–44 mg kg⁻¹) considering the very low concentrations of extractable Cd in the soil (< 1 mg kg⁻¹). Such values could arguably be recognised as hyperaccumulation as well. They are in the same order of magnitude as Cd concentrations found in *T. caerulescens* on mine sites in Great Britain (e.g. Baker *et al.*, 1994).

Data on multielement composition of *Thlaspi caerulescens* in natural conditions are scarce. Our results are apparently the first to demonstrate that plant/soil concentration factor is highest for Zn compared with other metals. This suggests that Zn might well be the actual target of hyperaccumulation in *T. caerulescens*. This result is seemingly difficult to reconcile with the 'inadvertent uptake hypothesis' which is one of hypotheses put forward to explain the raison d'être of hyperaccumulation (Boyd & Martens, 1993; Macnair, 2003).

Significant correlations between element concentrations in plants and in soil suggest that variation in mineral element concentrations of field-growing plants is, in part, of environmental origin. Thus, those populations growing in soil with above average concentrations of Mn and Zn also tend to have higher concentrations of these elements in the leaves. Foliar concentration of Mg also appears to be much dependent on soil conditions, being negatively correlated to soil pH. This may be due to a competitive interaction between Ca and Mg in the uptake process.

Genetic variation in mineral element composition

The present study demonstrates the existence of substantial stores of genetic variation of mineral element composition in *T. caerulea* in Luxembourg. This is a striking result, considering that all populations are located in a restricted geographic area and occur in the same ecological context. There is a two-fold variation range in Cd and Zn accumulation capacity in that region. These results stress that a single 'ecotype' of *T. caerulea* may contain substantial stores of genetic variation, thus implying that generalisations based on a single population or a limited number of genotypes must be taken with caution. A few studies had previously found genetic variation in mineral composition in *T. caerulea*, but all of them focused on Zn and Cd in very few populations from contrasting ecogeographic origins (Pollard & Baker, 1996; Meerts & Van Isacker, 1997; Escarré *et al.*, 2000; Lombi *et al.*, 2000; Roosens *et al.*, 2003).

The structure of genetic variation in metal accumulation is in line with the selfing breeding system of these populations. Riley (1956) calculated an outcrossing rate of *c.* 5% in *T. caerulea*. Koch *et al.* (1998) however, using allozymes, showed that outcrossing rate varied strongly depending on populations. Dubois *et al.* (2003) based on allozymes and P/O counts inferred that nonmetallicolous populations, including those from Luxembourg, had higher selfing rates compared to metallicolous populations. Founder effects may also promote population differentiation.

What is the ecological significance of the differences in metal accumulation existing among populations? A first hypothesis is that they have evolved in response to selection by local soil conditions. For instance, we might expect populations from low Zn soil to have evolved higher Zn uptake capacity. Two conditions should be fulfilled to conclude that genetic variation results from selection by local soil conditions: first phenotypic variation in mineral element concentrations in the field must be correlated to genetic variation assessed in uniform conditions (i.e. phenotypic variation has a significant genetic component); and second genetic variation must be correlated to local soil conditions. Correlations between field and glasshouse plants were not significant (Zn, $r = +0.54$; Cd, $r = +0.31$; Ca, $r = -0.04$; K, $r = +0.11$; Mg, $r = -0.04$; $n = 8$, all with $P > 0.05$; the other elements were not tested because they had no genetic variation). Correla-

tions between glasshouse plants and local soil (72 coefficients were calculated, i.e. eight elements in plants \times nine soil parameters) were also nonsignificant (data not shown). Thus, we have no strong evidence for selection on mineral element composition. However, this conclusion must be taken with caution, because of the low statistical power of the tests ($n = 8$ populations) and because the plants were cultivated in soil that is not close to natural soils. Mineral element composition might still have evolved in response to some other selective force (e.g. herbivory). Stochastic effects (genetic drift and founder effects) are an alternative hypothesis accounting for the genetic variation observed between populations. Low population size may result in frequent extinction of local populations, followed by recolonisation.

Cd–Zn correlation

This study is the first to show a genetic correlation between Cd and Zn accumulation within a restricted region. The very tight Zn–Cd correlation ($r = +0.83$, $P < 0.001$, Fig. 4) provides circumstantial evidence for a common transport system of these metals in the populations investigated. Assunção (2003a) investigated interactions between Zn, Cd and Ni in the uptake process in one population from Luxembourg. She found evidence for the existence, in that population, of a Zn transporter with a low affinity for Cd. The covariation between Cd and Zn might be due to genetic variation in the regulation of that transporter.

Relation between metal accumulation and plant size

Variation in Zn–Cd concentration was not significantly correlated to variation in biomass (Zn, $r = -0.06$ ns; Cd, $r = -0.09$ ns). There is thus no evidence for a genetic trade off between metal accumulation capacity and growth in those populations. It may thus be possible to select genotypes combining high metal extraction and high biomass, for the purpose of phytoremediation. Pollard & Baker (1996) reached similar conclusions for metallicolous populations from Great Britain.

Conclusions

Thlaspi caerulea is represented in Luxembourg by a large number of nonmetallicolous populations. There, the species occupies a well-defined ecological niche exhibiting a marked thermophilous and heliophilous behaviour.

Mineral element composition of field-growing plants is directly influenced by local soil conditions in the case of Zn, Mn and Mg. However, significant stores of genetic variation in mineral element composition and biomass have been detected, mostly occurring at the between population level. The maintenance of the hyperaccumulation property on normal soil demonstrates that hyperaccumulation and tolerance represent distinct properties that can evolve independently of

12 each other (Pollard *et al.*, 2002; Assunção *et al.*, 2003c). From a more applied point of view, populations on normal soil, with higher metal accumulation capacity and significant stores of genetic variation may represent useful materials for breeding programmes aiming at improving phytoextraction efficiency.

Nonmetallicolous populations of *T. caerulescens* in Luxembourg indisputably represent an important biological patrimony. Narrow ecological niche and low population size certainly make the species vulnerable. From a conservation point of view, our results indicate that a sufficiently large number of populations must be sampled to capture most of the genetic variation of biomass and mineral element composition. Careful management of road banks will be necessary to ensure survival of the species in the long term.

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